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14. ABSTRACT
The hypothesis of this proposal is that Latent TGF-beta Binding Protein 1 (LTBP1) is a pro-metastatic factor in breast cancer. To test this hypothesis we proposed three aims 1) Determine the utility of LTBP1 expression as a biomarker in human breast cancer 2) Determine the consequences of modulating LTBP1 levels on cell motility, invasion and metastasis in breast cancer cell lines 3) Investigate the pathobiological effects of gain and loss of LTBP1 in genetically engineered mouse models. Our most important findings in this cycle are 1) we have confirmed in independent datasets that elevated LTBP1 expression is associated with poor outcome in Basal and HER2 positive ER-negative breast cancers; in contrast, LTBP 2-3 are more highly expressed in luminal cancers and associated with better outcome; 2) highly metastatic mesenchymal-like triple negative breast cancer cells express elevated LTBP1 and specific TGF-beta ligands and receptors and the LTBP binding partners Fibronectin and Fibrillin; and 4) that metastatic mouse tumors C3(1)-Tag and MMTV-PYMT express elevated LTBP1. This was achieved by determining the expression of total LTBP1 and binding partners by qPCR in a large panel of cells and their metastatic variants and by lentiviral knock down.

15. SUBJECT TERMS
Cell Adhesion, Involution, Metastasis, Latent TGFbeta Binding Protein, Pregnancy-associated Breast Cancer

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INTRODUCTION: Latent TGF-beta binding protein 1 (LTBP1) sequesters TGF-beta within the extracellular matrix and is essential for integrin-mediated stretch activation of this cytokine. In previous reports we have documented and published that breast ducts are surrounded by LTBP1, bringing TGF-beta into close proximity to breast stem cells located in the basal epithelial layer. Our results showed that LTBP1 expression is suppressed during lactation but dramatically elevated at involution, a stage linked to increased risk for highly metastatic forms of pregnancy-associated breast cancer. TGF-beta acts as a tumor suppressor early in cancer formation but later on promotes metastasis. This proposal tests the hypothesis that elevated LTBP1 is a critical element in promoting metastasis by examining LTBP1 expression in breast cancer and testing whether altering LTBP1 levels affects invasion and metastasis in breast cancer cells *in vitro* and *in vivo*.

2. KEYWORDS: Cell-matrix Adhesion, Involution, Metastasis, Latent TGF-beta Binding Protein, Pregnancy-associated Breast Cancer

3. ACCOMPLISHMENTS:

- **What were the major goals of the project:**

Task 1) Determine the utility of LTBP1 expression as a biomarker in human breast cancer. (80 % complete)
Task 2) Determine the consequences of modulating LTBP1 levels on cell motility, invasion and metastasis in human and murine breast cell lines. (90% complete)
Task 3) Determine the pathological significance of gain and loss of LTBP1 in genetically engineered mouse models. (75% complete)

- **What was accomplished under these goals:**

Task 1. Determine the expression of LTBP1 in human and mouse breast cancers and breast cancer cell lines (80% complete).
LTBP1 is a secreted protein that integrates into the extracellular matrix and was therefore expected to be produced by neighboring basal or stromal cells. Previously, we reported the unexpected finding that LTBP1 is produced by luminal cells. This finding has consequences for our understanding of the mechanism of breast cancer spread as it indicates that luminal cells, which are considered to be the source of all different types human breast cancers, secrete a prometastatic soil. However, there are three other members of the LTBP family in addition to LTBP1, and as none have been studied in breast it is important to establish whether they act redundantly with LTBP1 or differ in their contribution to mammary biology and breast cancer. To provide a comprehensive understanding of the potentially interrelated roles of these proteins we investigated their expression by qRT-PCR analysis over the course of normal mammary development **(Fig. 1)** shows that LTBP3 is expressed in a similar temporal developmental pattern to that of LTBP1 being markedly elevated during involution. In contrast LTBP2 and 4 are expressed at extremely low levels and remain constant over the course of development.

To ascertain which cell types within the mammary gland express these LTBP genes we isolated mammary cells from normal mammary tissue and sorted them into the three major populations by fluorescent activated cell sorting (FACS) based on their CD49f and CD24 surface marker expression. Enrichment for basal, luminal and stromal subpopulations was confirmed by PCR analysis of mRNA for their specific intermediate filaments Keratin (K) 14, K8 and Vimentin respectively **(Fig. 2).** PCR analysis of LTBP mRNA expression established that Ltbp1, 1L and 1S are enriched in luminal cells as expected, Ltbp2 is expressed in basal and stromal cells, Ltbp3 is expressed in basal and luminal and Ltbp4 in stromal cells **(Fig. 2).**

Previously, we have shown by in silico interrogation of the Kaplan-Meier Plotter database that high Ltbp1 expression was associated with poor outcome in terms of both recurrence-free and distant metastasis-free survival (Report year 1, Fig. 1). This association was found in breast cancer overall but was most strongly significant for Basal and HER2 subtypes (Report year 1, Fig. 2). This year we have investigated an additional database (BreastMark). These data add confirmation that high LTBP1 expression shows a weak association between and poor outcome in terms of disease-free survival when breast cancer are considered as a whole and a strong significant association with particularly poor outcome within Basal and HER2 subtypes **(Fig. 3).**

In marked contrast, to the association between high LTBP1 expression and poor outcome, we found the opposite was true for other members of the LTBP family. High LTBP 2, 3 or 4 expression was associated with improved outcome in terms of RFS for breast cancer overall **(Fig. 4).** This association with better outcome is particularly true within the luminal subsets in terms of RFS **(Fig 5).**

A complex scenario was found in terms of DMFS **(Fig. 6):** High LTBP3 expression was associated with slightly better outcome in luminal and basal subtypes. High LTBP 2 and 4 expression was associated with worse outcome. High expression of any LTBP was associated with significantly worse outcome in HER2 breast cancer.

Previously we reported (Report year 1 Fig 3) that LTBP1 was expressed more highly in breast cancer cell lines classified as Basal B, HER2-positive and ER-negative which are thought to derive from breast cancers with the worst outcome that are prevalent in young women of reproductive age. We confirmed LTBP1 expression was higher in breast tumors of these types (Report 2 Fig 1). Moreover, we found differential isoform expression of LTBP1 short (1S) and long (1L) isoforms in particular types of human breast cancer cell lines (Report year 2, Fig 2): LTBP1S was expressed specifically in the mesenchymal subtype of triple negative breast cancer (Report year 2 Fig 3) that is associated with high motility and invasive gene signatures. We have interrogated databases of TNBC human breast tumor that distinguish subtypes (TNBCtype). This database cannot distinguish between the two isoforms having used probes that recognize both. Our results show that total LTBP1 is significantly more highly expressed in TNBC tumors compared to all others and there is a trend towards higher expression on average

within the mesenchymal stem-like subgroup, which is the most highly metastatic form with the worse outcome **(Fig 7).**
In contrast LTBPs 2-4 show the opposite of LTBP1 in terms of their expression levels in different types of breast tumors being more highly expressed in ER-positive tumors and in Luminal A subtypes **(Fig. 8).** Currently we are focusing efforts on solving issues concerning antigen retrieval to allow examination of LTBP1 expression in archival human breast cancers and normal tissue as controls. Our preliminary data on paraffin embedded human breast are shown in **Fig. 9.**

Conclusions: In conclusion these analyses show that LTBP1 correlated with absence of estrogen receptor and is associated with poor prognosis in breast cancer, particularly within Basal and Her2 subtypes. It is highest within TNBCs, particularly those of the most aggressive MSL subtype. These findings support the concept that LTBP1 is prometastatic factor. Given our previous finding of isoform specific expression of LTBP1S in TNBC cell lines it will be important to examine if LTBP1S expression correlated with specific TNBC tumor subtypes and survival. In contrast other LTBPs are more highly expressed in luminal A estrogen receptor-positive tumors and their high expression in this type of breast cancer is associated with better outcome. Higher expression of any LTBP is associated with worse outcome within the HER2 subtype of breast cancer.

Task 2 Determine the consequences of modulating LTBP1 level in breast cancer cell lines on motility, invasion and metastasis (90% complete).
Previously, we reported that highly metastatic variants of the TNBC cell line MDA-MB-231 express higher level of LTBP1 than their less metastatic parental cell line (Report year 2 Fig. 4). We generated a lentiviral knock down system that effectively reduced LTBP1 expression within this cell type using doxycycline inducible hairpins (Report year 2 Fig. 5 and 6). Cell proliferation, adhesion and migration remained unaltered (Report year 2 Fig. 7, 8, 9, 10). However, the ability of cells lacking LTBP1 expression to invade through matrigel in a transwell assay was severely compromised. This supports the concept that LTBP1 is proinvasive and raised two further questions. 1) Does it promote invasion by creating attachment to the extracellular matrix and/or through activation of TGF-beta. 2) Does it promote metastatic spread of breast cancer cells *in vivo*.

Ltbp1 is known to interact with a number of proteins. First, it can binds to the small latent complex of all three isoforms of Tgf-beta. Once secreted Ltbp1 binds to Fibronectin (Fn1) and then to Fibrillin 1 (Fbn1), thus it positions latent Tgf-beta in the ECM for subsequent release and activation either by integrin-mediated stretch activation or by enzymatic release. We wanted to elucidate how much of this apparatus breast cancer cells have. To begin to address the first question we have investigated a panel of breast cancer cell lines including MDA-MB-231 PCR for their mRNA expression of TGF-beta ligands **(Fig. 10a),** extracellular matrix binding partners **(Fig. 10b and c)** and TGF-beta receptors **(Fig. 10d).**

PCR for mRNA of the three isoforms of Tgf-beta showed that all breast cancer cells in our panel are producing one of other ligand **(Fig. 10a).** Interestingly, and to our knowledge previously unreported, Luminal type cell lines predominately expressed Tgf-beta3 while Basal A and Basal B cell lines express no Tgf-beta3 and instead expressed Tgf-beta1 and Tgf-beta2.

Next we examined if the cell lines were producing the ECM components for Ltbp1 to bind too. Intriguingly, only Basal B cell lines expressed Fibrillin 1. Fibronectin was detected more broadly across the panel but was strongly expressed in the Basal A and Basal B lines **(Fig. 10b).** This correlates with the Ltbp1S expression and suggests that these more highly invasive breast cancer cell lines have the capability to generate their own niche components to facilitate spread and colonization.

To further query the correlation between Ltbp1S expression and that of Fbn1 and Fn1 we performed PCR in the triple-negative breast cancer cell line panel **(Fig 10c).** All 5 Mesenchymal-like lines expressed both Fbn1 and Fn1 while only 2 out of 5 Basal-like cells show expression. This puts Mesenchymal-like TNBC cells in the position of being able to build their own cancer stem cell niche, supporting the concept that the seed of breast cancer generates its own prometastatic soil.

Last we examined the TGF-beta receptor profile and found that luminal cells express predominantly TGF-beta receptor 1 whereas Basal cells express TGF-beta receptor 2 and TGF-beta receptor 3 is predominantly expressed by Basal B cell lines **(Fig 10d).**

Collectively these data suggest that only Basal B cells generate a fibrillary matrix that will permit presentation and integrin-mediated stretch activation of TGF-beta and that they have the necessary receptors to respond.

To answer the second question concerning the ability of LTBP1 to facilitate metastasis we have established the procedure to introduce MDA-MB-231 cells harboring doxycycline-inducible sh-scr and sh-ltbp1 hairpins into the mammary fatpads of immunocompromised mice **(Fig 11).** We are monitoring this pilot set of animals and will be infecting and following a larger cohort by IVIS to track the effect of knocking down Ltbp1 on metastasis *in vivo* in order to complete Aim 2.

Task 3: Determine the pathological significance of gain and loss of LTBP1 in mice (75% complete).

Previously we reported problems in cloning human LTBP1 into pHIV-ZsGreen lentiviral vector and instead obtained a tagged version from a group at McGill. We are in the process of constructing the transgene. In the mean time we have determined the mRNA expression levels of all LTBPs within four different mouse mammary models **(Fig. 12A).** MMTV-Wnt1, MMTV-neu, MMTV-PYMT, C3(1)-Tag compared to normal FVBN controls. Intriguingly, Ltbp1 is elevated in the metastatic C3(1)-Tag and MMTV-PYMT strains and downregulated in the poorly metastatic MMTV-neu strain. Ltbp4 is markedly

downregulated in all tumors. Ltbp2 and 3 are modestly increased. From these analyses we now think that C3(1)-Tag model would be a good one to explore the role of LTBP1 in tumor progression. This mouse strain resembles human DCIS and 20% of mice progress to show a similar pattern of metastasis to that seen in humans. We have harvested primary tumors from PYMT mice and establishing protocols to efficiently infect these primary MECS with lentiviral knock down shRNA hairpins to determine the effects of modulating LTBP1 on tumor progression. We have checked for expression of Ltbp1 I the PYMT tumors by crossing these mice to a reporter strain *Ltbp1-lacZ* where beta-galactosidase is produced under the control of the Ltbp1 promoter. Intriguingly, Ltbp1 is seen at the pushing margins of highly invasive segments of these tumors **(Fig 12B).**

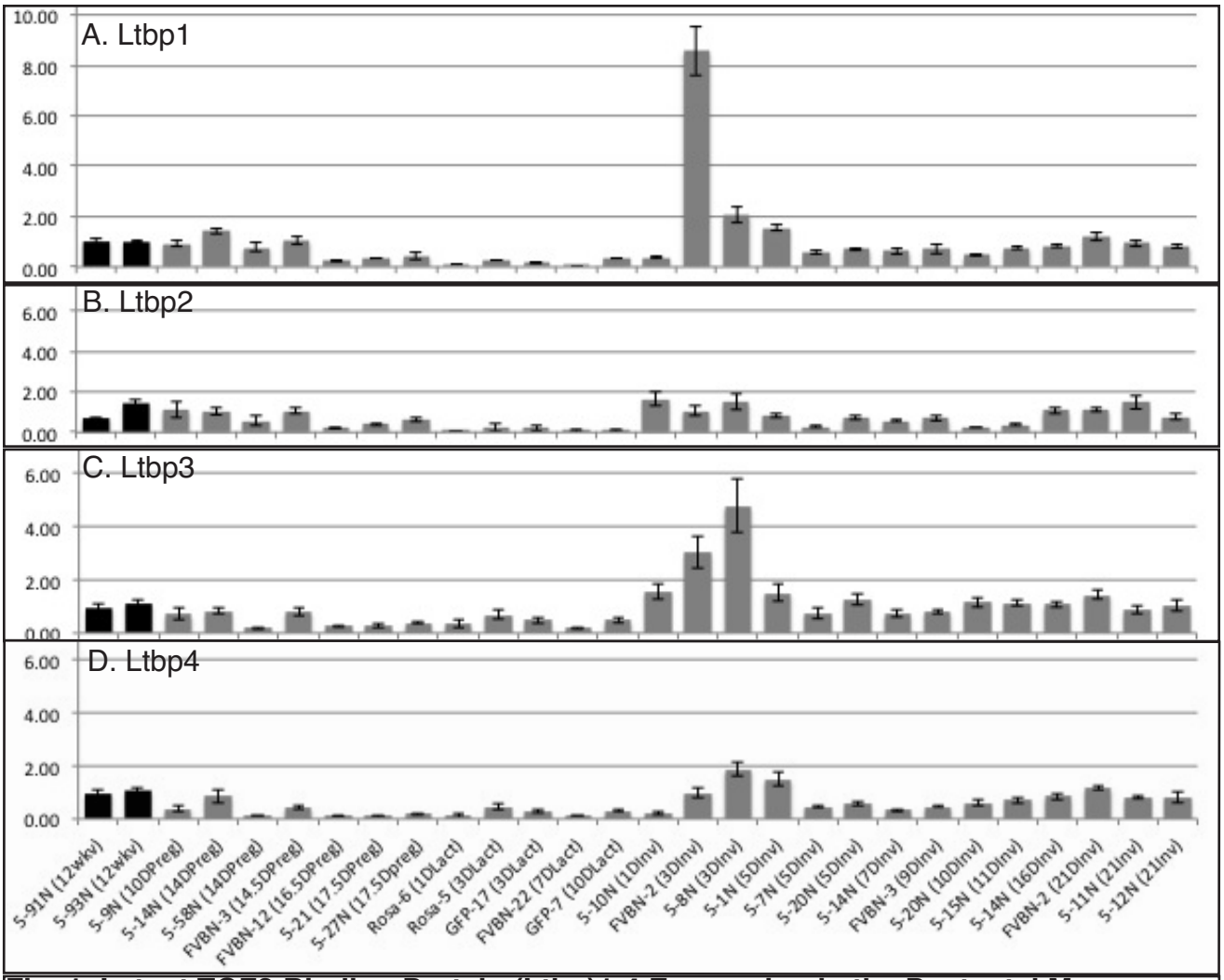


Fig. 1. Latent TGFβ Binding Protein (Ltbp)1-4 Expression in the Postnatal Mammary Gland.

(A) Total RNA from mammary gland tissues, harvested from various developmental time points (12-week virgin (wkV), 10, 14.5 and 17.5 days of pregnancy (DPreg), after 3, 7 and 10 days of lactation (DLact), days of involution (Dinv) days 3, 5, 7, 9, 10, 11, 16 and 21, was reverse transcribed and subjected to qRT-PCR. Ltbp mRNA were normalized to β2-microglobulin expression and plotted as levels relative to tissue from 12-week-old virgins. Error bars indicate standard deviation of the cycle threshold (Ct) values (n=4). Numbers below indicate the specific mice.Ltbp1 mRNA expression is highly elevated during early involution peaking 3 days after forced pup weaning. (B) Ltbp2 shows a low constant level of expression. (C)Ltbp3 is elevated during early involution peaking 3 days after forced pup weaning (D) Ltbp4 is relatively constant throughout mammary development.

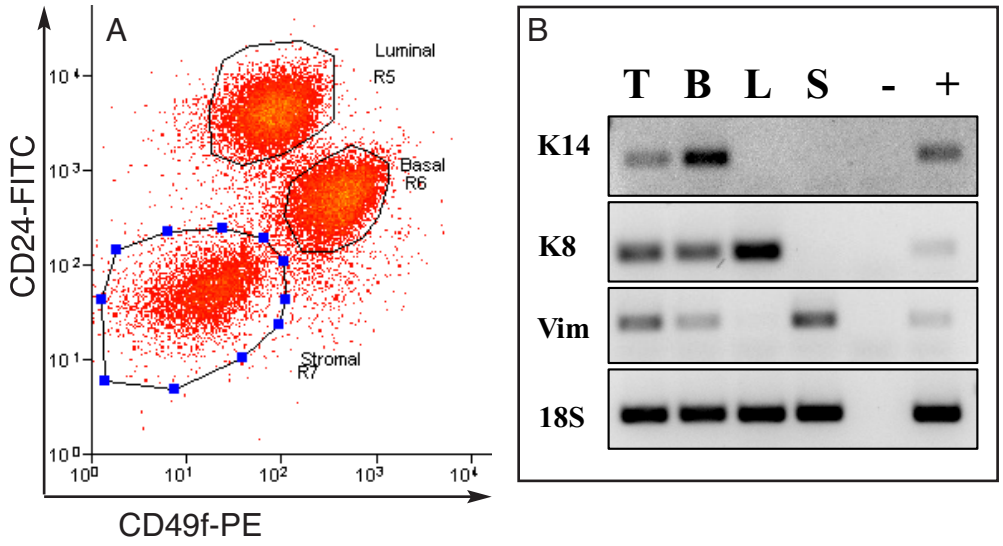
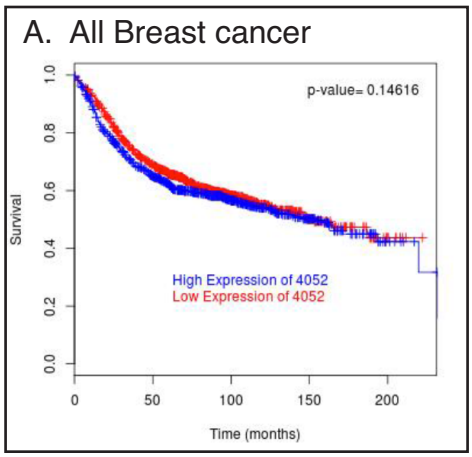


Fig. 2. Ltbp Expression in Mammary Cell Subpopulations. (A) Basal (B), Luminal (L) and Stromal (S) mammary subpopulations: gated on the basis of CD24 and CD49f marker expression by FACS.

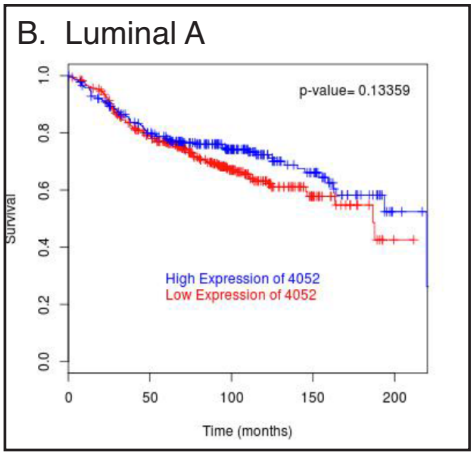
(B) PCR characterization of mRNA expression of Keratin 14 (K14), (K8) and vimentin (Vim) markers within total MECS (T) and basal (B), (L) luminal and (S) stromal cell subpopulations as gated in (A). 18S rRNA served as a loading control. Control (-) indicates the negative control with no cDNA template added and (+) indicates the positive control cDNA derived from a MMTV-Wnt tumor.

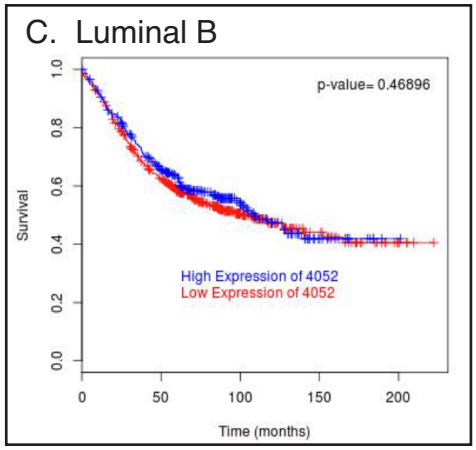
(C) PCR characterization of mRNA expression for Ltbp1-4 as indicated within the same subpopulations characterized above. Note Ltbp1, 1L and 1S are enriched in luminal cells. Ltbp2 is expressed in basal and stromal cells, Ltbp3 is expressed in basal and luminal and Ltbp4 in stromal cells

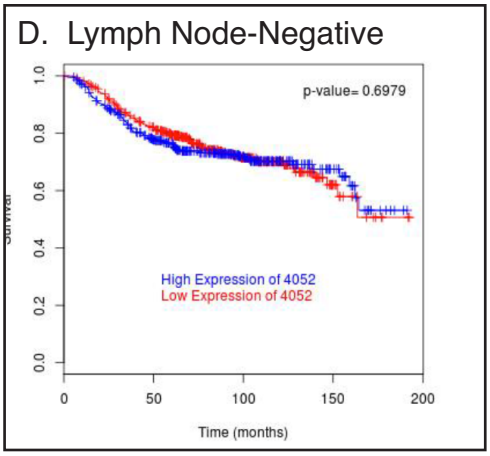
Fig. 3. Analysis of LTBP1 expression and Disease-Free Survival (DFS) in BreastMark Database

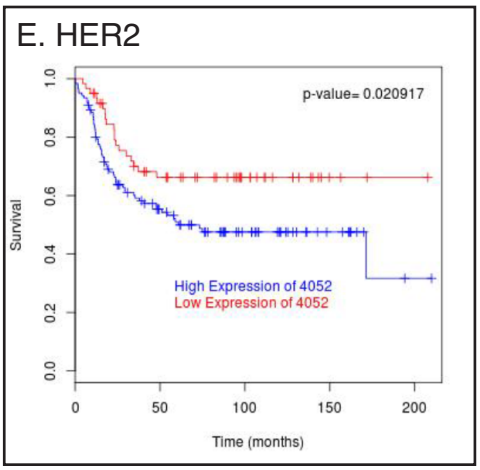


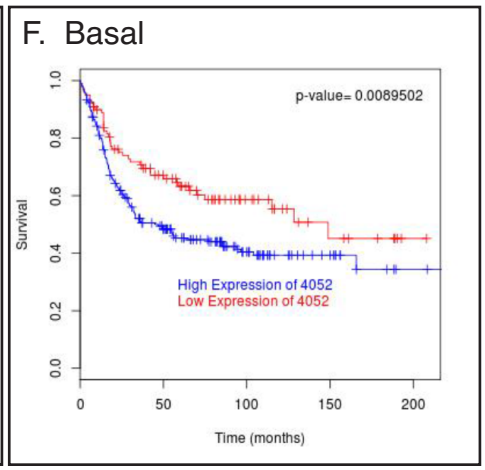
High LTBP1 expression is associated with worse outcome (DFS) inbBasal, HER2 & Lymph Node (LN)-positive breast cancers. Association between LTBP1 expression and DFS was assessed using the online BreastMark tool that divides patients at the median for high (blue) and low (red) LTBP1 expression. N=number of samples, HR=Hazard Ratio and P value are as indicated. No significant association was found in Luminal A, Luminal B or LN-negative breast tumors (B-D). However, high LTBP1 expression identifies a subset of patients with significantly worse outcome in Basal and HER2 subtypes (E-G) and those with LN-positive tumors.

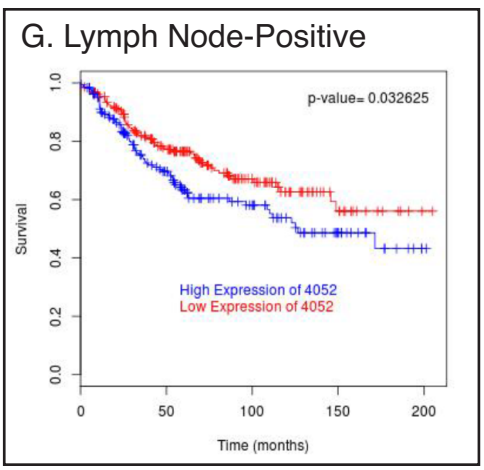




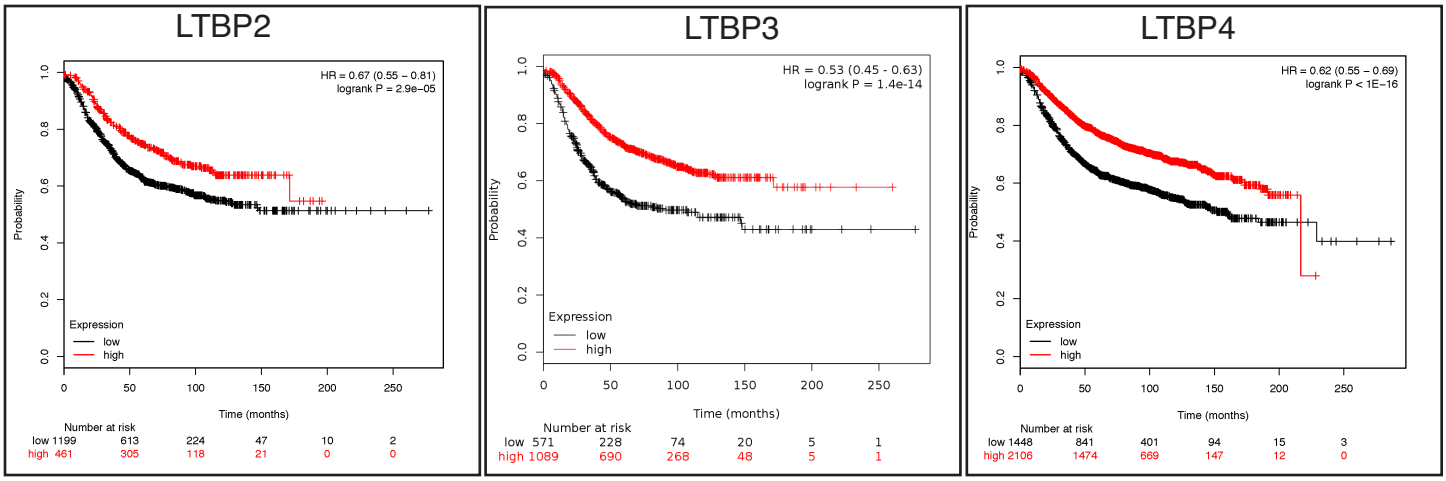








KMplotter - RFS (red=high expression)



Breastmark - DFS (blue= high expression)

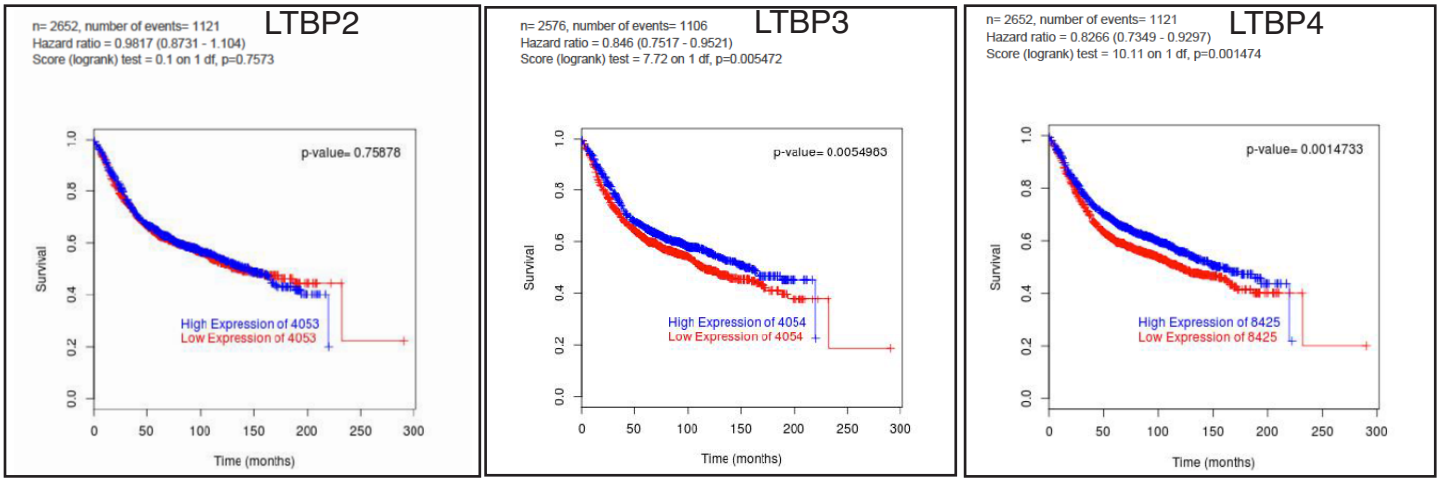


Fig. 4 High LTBP2, 3 and 4 Expression is Associated with Better Outcome in Breast Cancer Overall.

A) Relapse-Free Survival (RFS) data were plotted using the online assessment tool Kaplan-Meier Plotter (kmplot.com) to divide breast cancer patients into high (red) and low (black) expression cohorts on the basis of best-fit criteria as indicated below the graph.

B) Disease-Free Survival (DSF) data were plotted using the online assessment tool BreastMark to divide breast cancer patients according to the median into high (blue) and low (red) expression cohorts. The results show that high LTBP2, 3 and 4 expression is correlated with better survival. Hazard Ratio (HR) and P value are as indicated.

Fig. 5. High LTBP 2, 3 and 4 Expression (red) Is Associated with Better Outcome (RFS) in Luminal Breast Cancer.

RFS in breast cancer patients plotted using Kaplan-Meier Plotter (kmplot.com) selecting for best-fit to divide the cohort into tumors with high (red) and low (black) LTBP 2, 3 and 4 expression. Hazard Ratio (HR) and P value are as indicated. The results show that in contrast to LTBP1, which correlates with bad outcome, high expression of LTBP 2, 3 and 4 is associated with better outcome in Luminal breast cancers.

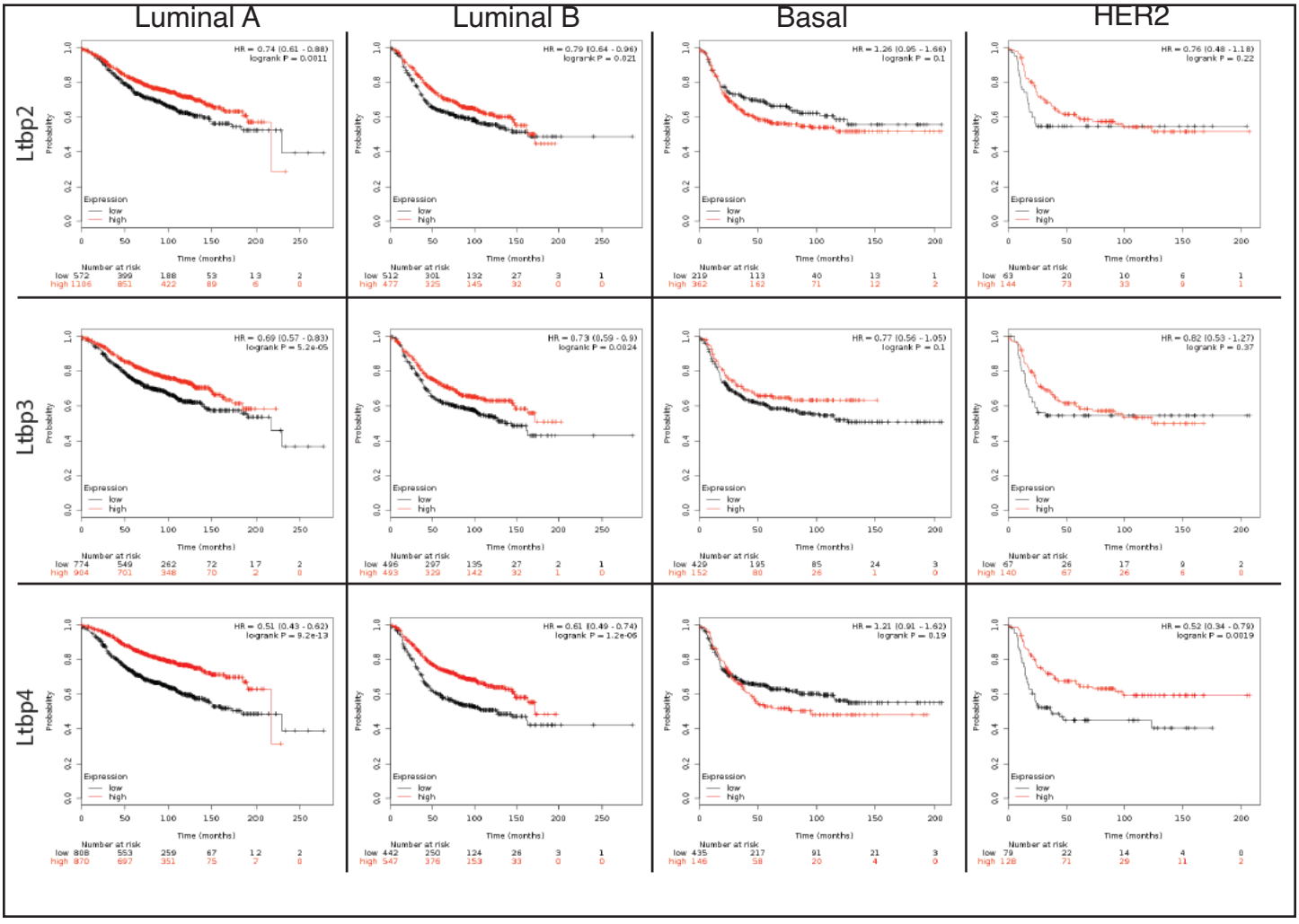


Fig. 6. High Expression of any LTBP is Associated with Worse Survival for Her2 Breast Cancer Patients

A) Distant Metastasis-Free Survival in breast cancer patients plotted using Kaplan-Meier Plotter (kmplot.com) selecting for best-fit to divide the cohort into tumors with high (red) and low (black) LTBP 2, 3 and 4 expression. Hazard Ratio (HR) and P value are as indicated. The results show that high LTBP2 and 4 expression has no statistically significant effect in luminal cancer whereas high LTBP3 is protective. In contrast high expression of any LTBP is associated with worse outcome for Her2 breast cancer patients.

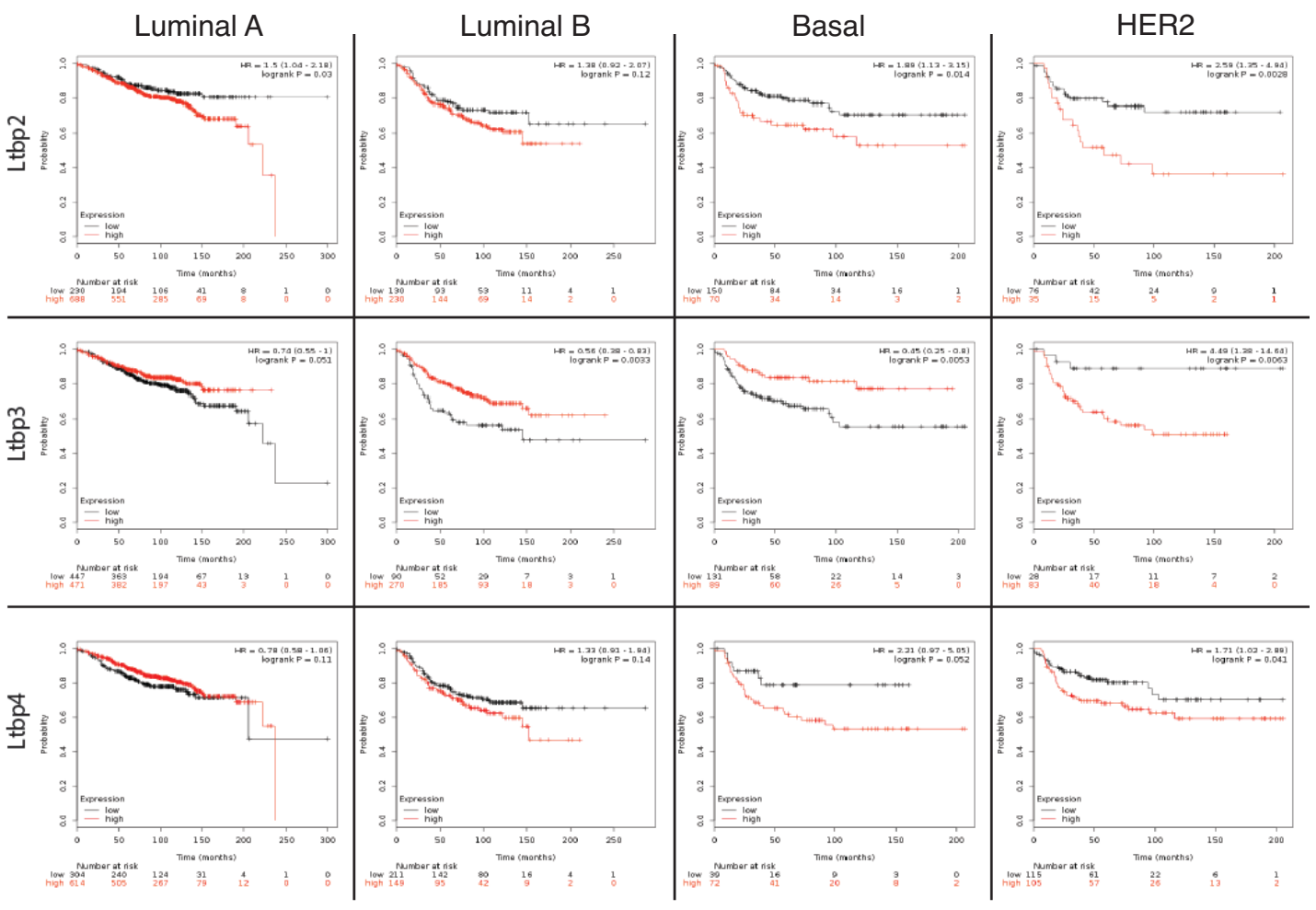


Fig. 7 LTBP1 Expression is Higher in Triple Negative Breast Cancers.
(A) LTBP1 expression in TNBC versus all other breast cancers in the TGAC database ****= p<0.0001 and (B) Hess et al. C) LTBP1 expression in TNBCtype database http://cbc.mc.vanderbilt.edu/tnbc/index.php) BL1:Basal-like1, BL2:Basallike2, IM:Immunomodulatory, M:Mesechymal, MSL:Mesenchymal-stem-like,LAR:Luminal Androgen Receptor,UNS:Unassigned. There is a trend towards higher expression within the mesenchymal stem-like subgroup.

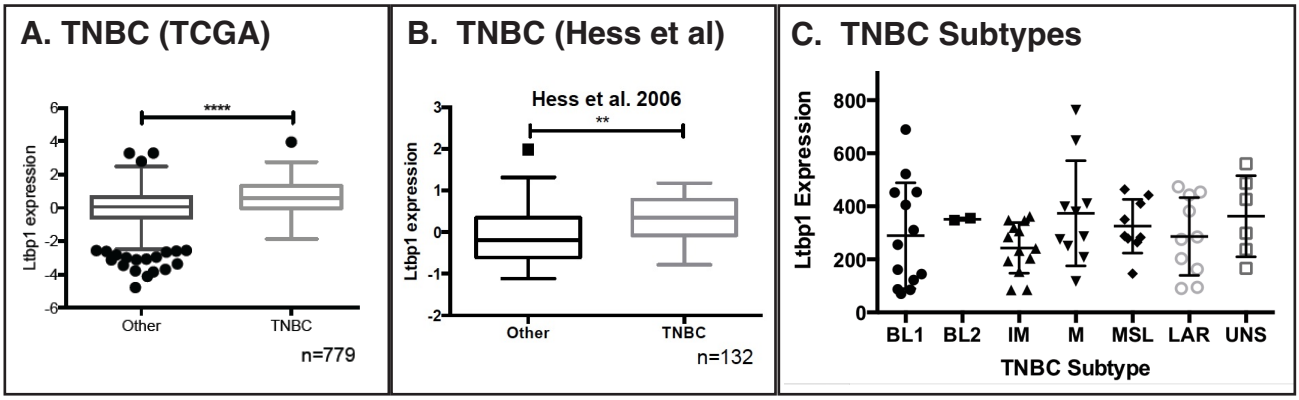
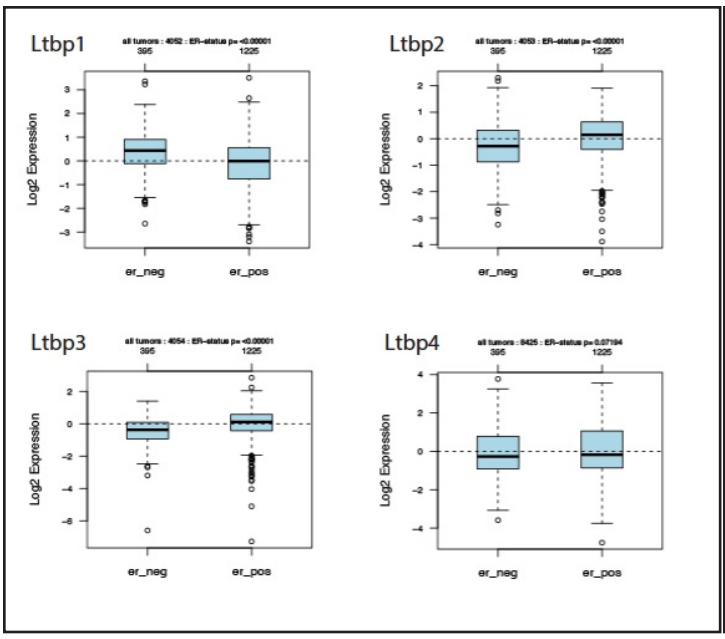


Fig. 8. Analysis of the Relative Levels of LTBP 1-4 Expression in Different Types of Human Breast Tumors
As shown in (A) LTBP1 is more highly expressed in estrogen receptor (er)-negative than er-positive tumors. In contrast LTBPs 2, 3 and 4 show the opposite trend.
(B) Likewise LTBP1 is more highly expressed in basal and HER2-positive subtypes and lower in luminal subtypes. In contrast LTBP 2, 3 and 4 are more highly expressed in Luminal A subtypes.

A



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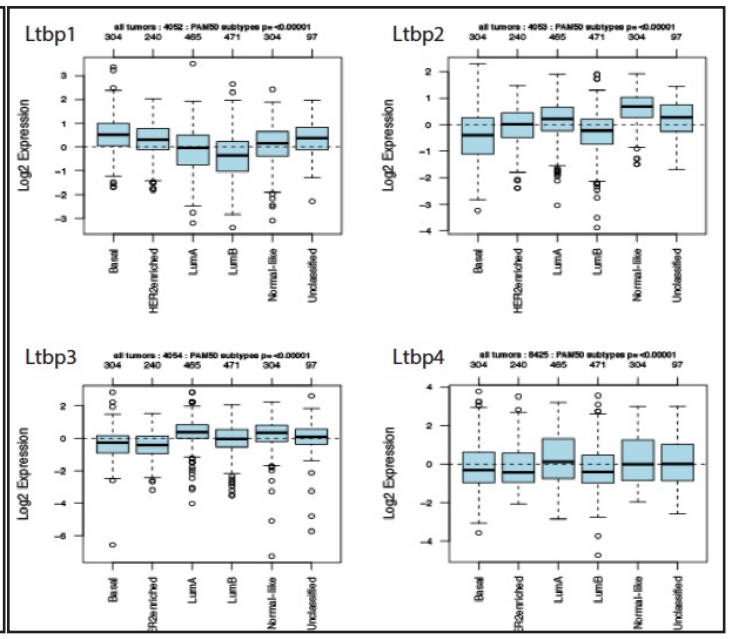


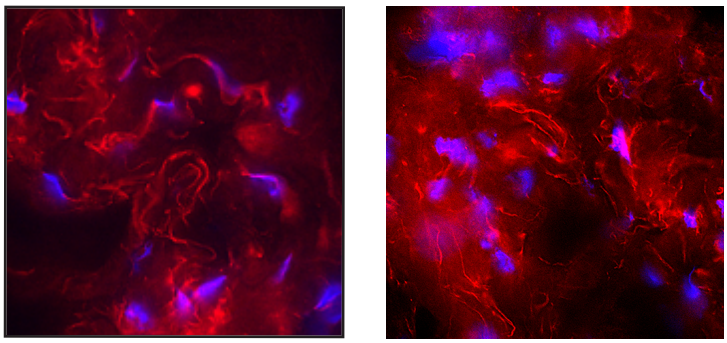


Fig. 9: Staining of LTBP1 in Elastic fibers surrounding Human Breast Tissue.
LTBP1deteted using Rabbit anti-Ltbp1 primary antibodies and rhodamine coupled secondary (red), nuclei counterstained with DAPI (blue).

Fig. 10. Breast Cancer Cell line Expression of LTBP1 ECM, TGF-beta Partners and Receptor
PCR analysis of the mRNA expression of the potential binding partners of LTBPs in different subtypes of breast cancer cell lines (Luminal= MCF7,T47D, ZR751, MDA-MB-361, ZR7530, AU565) (Basal A = HCC1569, HCC1954, MDA-MB-468) and (Basal B= BT549,HS578T, MDA-MB-231) as indicated. Gapd serves as a cDNA control.
a) TGF-beta ligands (TgfB1, TgfB2, TgfB3). Note TgfB2 in Basal B and TgfB3 in luminal cell types
b) The extracellular matrix partners Fibrillin1 (Fbn1) and Fibronectin (Fn1)
c) Fibrillin and Fibronectin expression in different TNBC subtypes as indicated. Note Basal B and the MSL subtype of TNBC cell lines express both ECM binding partners.
d) TGF-beta receptor expression (Tgfbr1, 2, 3).

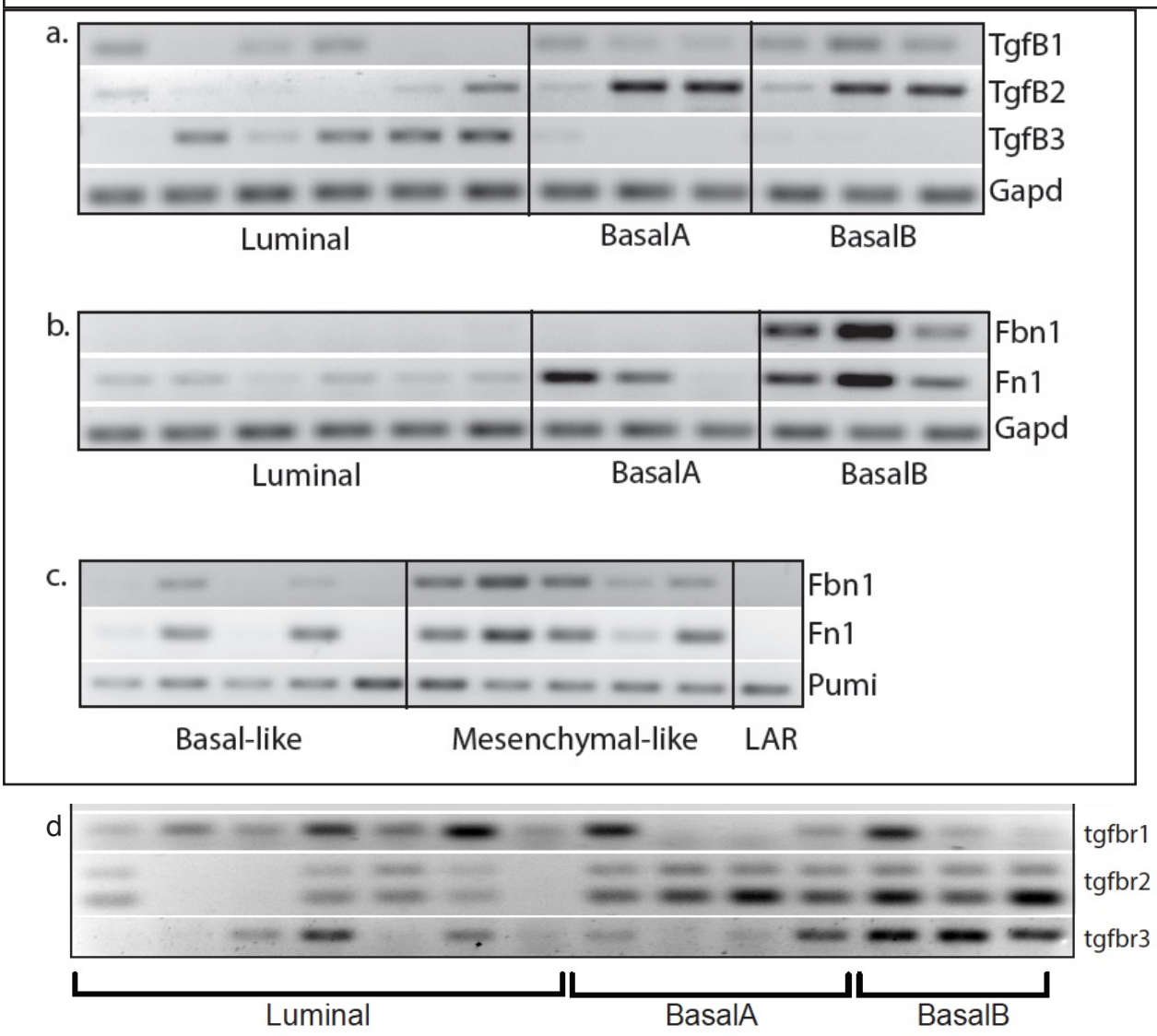


Fig. 11. Pilot Metastasis Assay for TNBC Cell Lines with Lentiviral Ltbp1 Knock-down

MDA-MB-231 cells were infected with lentivirus driving expression of sh-scr or sh-ltbp1 hairpins together with a luciferase cassette then injected into the mammary fatpads of SCID recipients. The cells were detected by in vivo imaging for luciferase expression (IVIS). We are currently monitoring these mice weekly for metastatic spread and infecting a larger cohort.

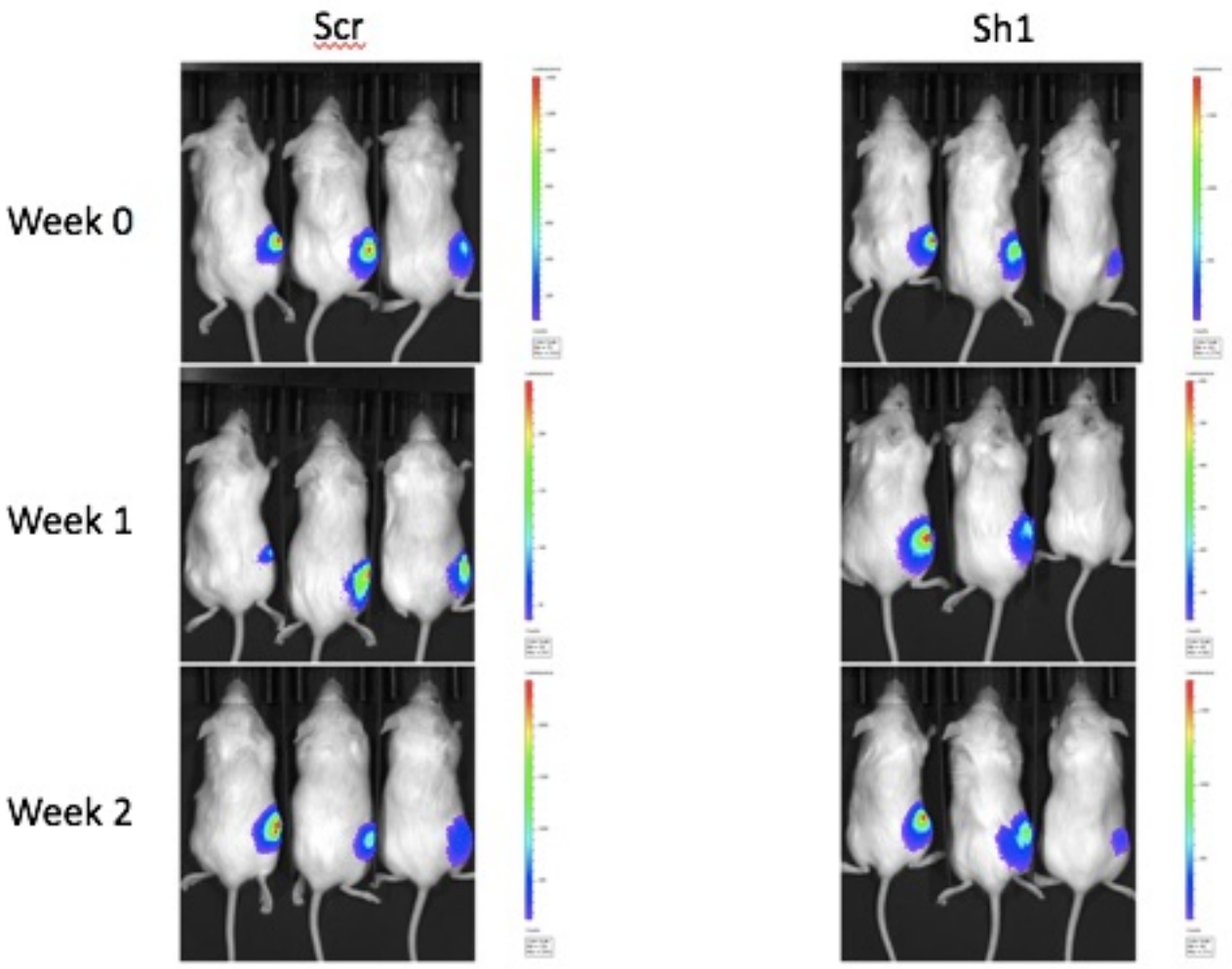
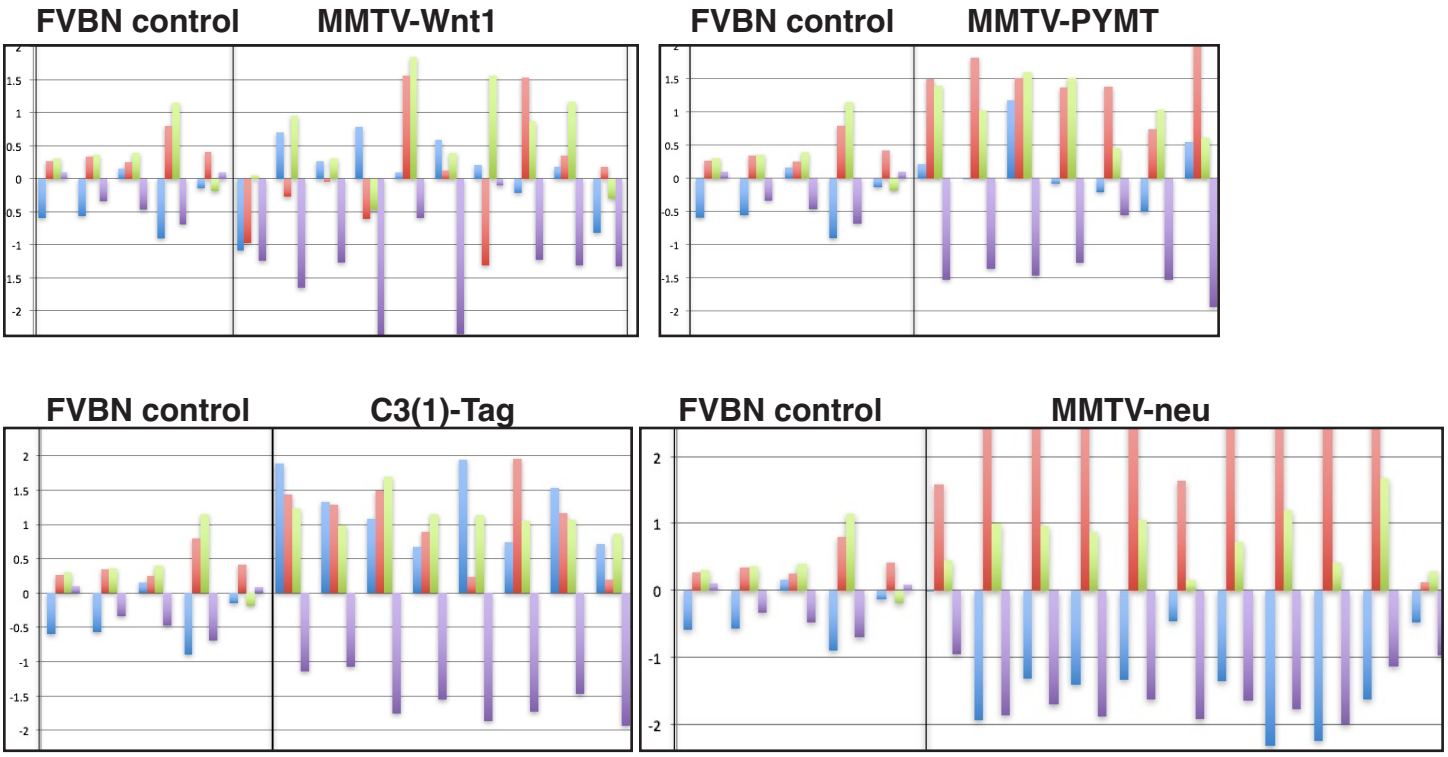
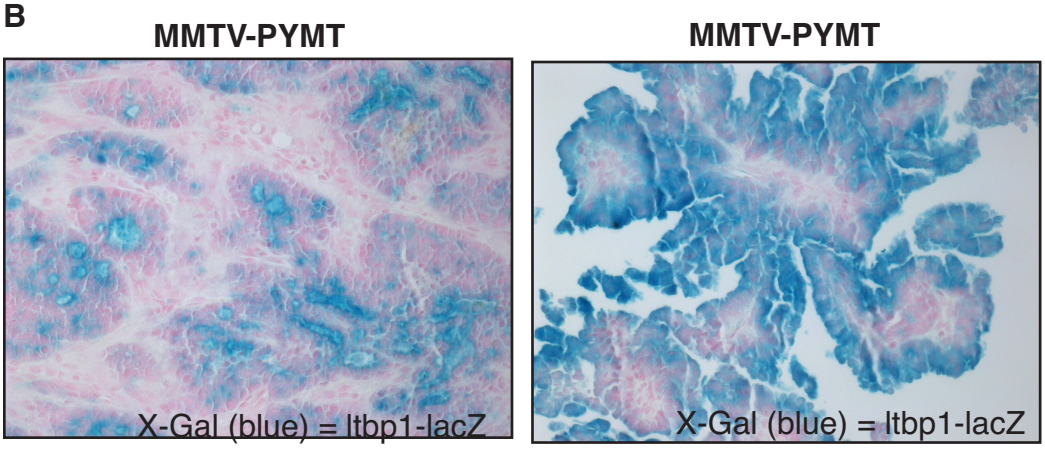


Fig. 12. Expression of Ltbp1-4 in Mouse Mammary Tumor Models
(A) qRT-PCR analysis of Ltbp1 (blue), Ltbp2 (red), Ltbp3 (green), and Ltbp4 (purple) mRNA expression in four different transgenic mouse mammary tumor models: MMTV-Wnt1, MMTV-neu, MMTV-PYMT,C3(1)-Tag compared to normal FVBN controls. Note Ltbp4 is downregulated in all tumors; Ltbp1 is elevated in the metastatic C3(1)-Tag and MMTV-PYMT strains and downregulated in the poorly metastatic MMTV-neu strain.
(B)Ltbp1-lacZ reporter expression detected by X-Gal staining (blue) in sections of a MMTV-PYMT tumor. Note the luminal expression within differentiated regions (left panel) and expression on the pushing margins of highly invasive regions (right panel)

A



B



- **What opportunities for training and professional development has the project provided.**

1. Dr. Chandramouli has acquired skills in shRNA Lentiviral knock down
2. Dr. Chandramouli has acquired skills in survival surgery and mammary transplantation and IVIS tracking of breast cancer cell dissemination.

- **How were the results disseminated to communities of interest**

1. Dr. Cowin presented this work as a poster at the GRC on Mammary Gland Biology June 2016
2. This work was presented to our scientist breast cancer survivor advocate: Dr. Cara Gottardi, Northwestern University.

- **What do you plan to accomplish during the next reporting period to accomplish the goals and objectives**

1. Complete the testing of human MDA-MB-231-LM2 cell lines lentivirally expressing LTBP1 hairpins for their ability to metastasize *in vivo*
2. Test for their ability of LTBP1 hairpins to reduce metastasis in MECS from MMTV-PYMT and C3(1)-Tag.

4. IMPACT

- **What was the impact on the development of the principal discipline of the project.**
- Our results have shown that high levels of LTBP1L occur in aggressive forms of basal and HER2 positive breast cancer, and that higher levels identify patients with particularly poor outcome within these groups. LTBP1S is highly expressed within the mesenchymal-like subset of TNBC cell lines and these cells appear to be the only forms of breast cancer that has all the apparatus to connect LTBP-TGF-beta complex to the ECM, activate and respond to it. This has pioneered a new field in breast cancer research since there are no studies besides our own on LTBP1. Our unpublished data reported herein suggest that other LTBP family members are associated with good outcome and therefore it is likely of significance that LTBP4 is downregulated in all murine breast cancer models and that LTBP1 is upregulated in metastatic models but downregulated in benign models. Our in vitro studies support the concept that LTBP1 aids cell invasion.

- **What was the impact on other disciplines**

Our in vitro studies support the concept that LTBP1 is proinvasive in breast and as it is expressed in other tissue this could be relevant in many types of carcinoma. Our work has also illuminated potential roles for LTBP1 in the physiology of lactation and in maintain stem cell viability during developmental periods of intense autophagy during breast involution.

- **What was the impact on technology transfer**

Nothing to report

- **What was the impact on society beyond science and technology**

Nothing to report as yet but linking breast development factor to breast cancer risk opens the door to preventative strategies linked to reproductive history.

5. CHANGES/PROBLEMS

- **Delays in approach and reasons for delay**
  We are delayed in generating the MMTV-LTBP1 transgenic due problems in constructing the transgenic cassette.

- **Actual anticipated problems or delays and actions or plans to resolve them**
  Nothing to report

- **Changes that had a significant impact on expenditures –** There was premature departure of one post-doctoral fellow from the project due to her need to relocate for family reasons this lead to a surplus at the end of year 3 that has been rebudgeted as a no cost extension to allow completion of the experiments.

- **Significant changes in use or care of human subjects, animals, biohazards or select agents** - Nothing to report

6. PRODUCTS

- **Publications, conference papers and presentations**

- ***Manuscripts:*** We are currently writing up the work detailed in Task 1 and 2 above to be submitted as a manuscript to Breast Cancer Research.

- ***Books etc:*** Nothing to report

- ***Other publications, conference papers and presentations***

1. Dr. Cowin presented this work at the GRC on Mammary Gland Biology, June 2016. ***Acknowledgement of Federal Support:*** YES

- **Website(s) or other internet site (s):** Nothing to report

- **Technologies or Techniques:** Nothing to report

- **Inventions, patent applications and/or licenses:** Nothing to report

- **Other products:**
  - **Research material:** Generation of inducible lentiviral LTBP1, LTBP1L and LTBP1S hairpin constructs

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Individuals working on the project:

Name	Pamela Cowin
Project Role	P.I.
Research Identifier	
Nearest person month worked	12
Contribution to project	Directed research
Funding Support	DOD BC123572 20%

Name	Anupama Chandramouli
Project Role	Postdoctoral fellow
Research Identifier	
Nearest person month worked	6
Contribution to project	Performed work on Aim 2
Funding Support	None

- **Has there been a change in the active support of the PI or senior key personnel since the last reporting period**

P.I. Dr. Pamela Cowin – No change

Postdoctoral Fellow – Dr. Catina Crismale Gann and Carrie Oliver were replaced by Dr. Chandramouli

- **What other organizations were involved as partners?**

None

8. SPECIAL REPORTING REQUIREMENTS N/A

9. APPENDICES

None